REMARKS

The Invention.

The present invention provides modified forms of pullulanase which maintain the ability to catalyze the hydrolysis of an alpha-1,6-glucosidic bond, compositions which comprise the modified pullulanase, methods of making the modified pullulanase and methods of using the modified pullulanase, especially for the saccharification of starch.

Status of the Application.

Claims 5-10, 12, 14, 15, 27-40 and 52-66 are pending in the application.

Claims 5-7, 9, 10, 12, 14, 15, 27-29, 31-40, 52, 53 and 55-66 are rejected. Claims 8, 30 and 54 are objected to.

Claims 12, 39 and 40 have been amended to state more clearly what Applicants believe is the subject matter of the invention. Applicants assert new matter has not been introduced by the amendment.

35 U.S.C. §112, second paragraph.

Claims 39 and 40 are rejected under 35 USC §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Specifically, the Examiner asserts that it is not clear whether the composition is made up of "the truncated pullulanase of claim 27" or whether the composition comprises a truncated pullulanase that is at least 60% or 80% in its length. Applicants have amended Claims 39 and 40 to more clearly state that it is the composition that comprises 60% or 80% of the truncated pullulanase.

Withdrawal of the rejection is respectfully requested.

35 U.S.C. §103.

The Examiner has rejected claims 5–7, 9-10, 12, 14, 15, 27–29, 31–40, 52–53, 55-61 and 63–66 as allegedly obvious. The Examiner has cited at least one of three references in the §103 rejections. Applicants initially present a summary of the cited art.

Deweer, et al. (US Pat. No. 6,074,854)

The Examiner cites Deweer *et al.* as teaching pullulanase from a Gram positive bacteria, methods of making the recombinant enzyme, compositions either in the solid or liquid form and compositions comprising additional enzymes. However, the Examiner correctly recognizes that there is no teaching to modify the pullulanase by way of deletion of about 100, 200 or 300 N-terminal amino acids. Furthermore, there is no suggestion in Deweer *et al.* to modify any pullulanase, let alone a *Bacillus* pullulanase.

There is nothing in Deweer *et al.* that would suggest to or motivate the skilled artisan to truncate the *Bacillus* pullulanase or to combine its teachings with McPherson *et al.* or Albertson *et al.*

McPherson et al. (Biochemical Soc. Trans., (1988) 16(5):723-724)

The Examiner cites McPherson *et al.* as teaching proteolytic digestion, computer-based sequence analysis, and that the long N-terminal region lacks any catalyzing site. See the paragraph bridging page 4 of the Office Action.

The Examiner correctly notes that McPherson *et al.* does not teach a *Bacillus* pullulanase; it teaches a pullulanase from *Klebsiella pneumoniae,* a **gram-negative** rod-shaped bacteria. As further noted by the Examiner, McPherson *et al.* illustrates the relative position of 5 conserved regions with alpha-amylases from *Bacillus* or *Streptomyces*, both gram-positive organisms, or a fungus. McPherson *et al.* provides no comparison with a *Bacillus* pullulanase nor a suggestion that a pullulanase from a gram-positive organism shares similarity with a pullulanase from a gram-negative organism and that it would be similarly affected by truncation. In fact, McPherson *et al.* provides that there were "[n]o extensive homologies were detected with any protein sequences in the database". See page 723, first column, second paragraph. Thus, not

only does McPherson *et al.* fail to suggest or motivate the skilled artisan to combine the teachings to obtain a truncated pullulanase from a *Bacillus* species it actually teaches away.

The pullulanase taught by McPherson *et al.* demonstrated no extensive homology with other proteins. See page 723, column 1, second paragraph. The pullulanase of McPherson *et al.* has approximately 1100 amino acid residues. This is larger than the 957 amino acid pullulanase from *B. deramificans* (including its signal sequence). Furthermore, the conserved "amylase" sequences are not provided by McPherson *et al.* so an alignment is not easily accomplished, if at all. Thus, Applicants contend the combination of references does not contain a sufficient teaching of how to obtain a truncated pullulanase from a *Bacillus* species.

In addition, Applicants note that McPherson *et al.* at best teaches that there may be some length of "the N-terminal region of pullulanase that has no defined catalytic function" for the *Klebsiella* pullulanase. However, it is silent on whether or not other truncated pullulanases, in particular *Bacillus* pullulanases, would possess similar properties, characteristics or corresponding increases in activity. McPherson *et al.* notes that the results were for "this variant" and not for any other *Klebsiella* variant. Thus, there is no indication that a skilled artisan would have a reasonable expectation of success with any other *Klebsiella* variant or, more generally, with any pullulanase.

Finally, the Examiner asserts that McPherson *et al.* teaches "the modification of deleting nearly 170 amino acid residues from the amino terminal end which leads to approximately 30% higher activity than that of the native enzyme." Applicants respectfully note that the results were preliminary and were based on a single substrate using an unspecified assay under unspecified conditions. See page 273, second column, first full paragraph. The substrate used was starch and therefore the assay used (which was not disclosed) could have been measuring the release of reducing sugars. McPherson *et al.* even state that the assay results only "suggest" that the debranching activity had increased. It is equally plausible that the amylolytic activity

(and not the debranching activity) had increased resulting in increased reducing sugars. Once again, McPherson *et al.* fails to teach critical parameters such as the exact assay used as well as the pH, temperature, time, etc. needed to replicate the work. Therefore, McPherson *et al.* fails to provide the skilled artisan with a reasonable expectation of success.

There simply is no motivation to combine McPherson *et al.* with any of the cited art.

Albertson et al., (Biochimca et Biophysica Acta, vol. 1354 (1) (1997):35-39)

The Examiner cites Albertson *et al.* (Cloning and sequence of a type I pullulanase from an extremely thermophilic anaerobic bacterium, Caldicellulosiruptor saccharolyticus. Biochimca et Biophysica Acta, vol. 1354 (1) (1997):35-39) as teaching the modification of a pullulanase isolated from *C. saccharolyticus*, wherein nearly 381 nucleotides from the 5' region of the cDNA encoding a pullulanase and that the deleted amino acid sequence is not essential for either activity or thermostability. See page 4 of the Office Action.

Applicants note that *C. saccharolyticus* is an extreme thermophile, i.e., the organism is found in conditions of high heat. There is no teaching or suggestion that pullulanases from microorganisms that are not thermophiles would possess the same properties or characteristics.

The enzyme most closely related to the pullulanase of *C. saccharolyticus* shares only about 35% identity with it. See page 36, column 2 of Albertson *et al.* Furthermore, Albertson *et al.* teaches that "there is no three-dimensional structure for a pullulanase." See page 238, first column, fourth paragraph.

The truncated enzyme of Albertson *et al.* has a truncation of 95 amino acids (see page 38, top of column 2) from its N-terminus, not the about 381 amino acids as indicated by the Examiner. See page 5 of the Office Action. The 381 nucleotide deletion apparently is in reference to recombinant plasmid pNZ1038 which has non-coding sequences in the 5' region of the DNA fragment. Not only does this fail to

suggest the required amino acid deletions but it also does not lead one of skill in the art to expect that larger deletions would be tolerated.

Albertson *et al.* also purports to identify the conserved regions which define the enzymatically active portion of the enzyme (see page 38, column 2, first full paragraph). They further state that "any regions of common amino acid sequence are likely to be important in elucidating the essential amino acid sequences critical for the enzyme (i.e. those sequences that form the essential enzyme 'signature'." However, the present inventors have identified two further conserved sequences which are located upstream (i.e., closer to the N-terminal) than those identified in Albertson *et al.* These are the Y and VWAP regions, which indicate the limits of amino acid truncations in the N-terminal of pullulanases in general." These regions are described in the passage spanning pages 11 and 12 of the present application. Albertson *et al.* fails to identify these regions nor provide any guidance regarding the retention of these sequences in a truncated pullulanase.

Claims 5–7, 9-10, 14, 15, 27–29, 31–40, 52–53, 55-61 and 63–66

The Examiner has rejected claims 5–7, 9-10, 14, 15, 27–29, 31–40, 52–53, 55-61 and 63–66 as allegedly obvious over the combination of Deweer, *et al.* (US Pat. No. 6,074,854) in view of McPherson *et al.* (Biochemical Soc. Trans., (1988) 16(5):723-724) or Albertson *et al.*, (Biochimca et Biophysica Acta, vol. 1354 (1) (1997):35-39). Applicants respectfully traverse the rejection.

An essential requirement for a *prima facie* case of obviousness is whether a person skilled in the art would be **motivated** to modify the references to arrive at the **claimed invention**. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598-99 (Fed. Cir. 1988) and *In re Jones*, 21 USPQ2d 1941, 1943 (Fed. Cir. 1992). In particular,

> "the examiner must show *reasons* that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the *claimed invention*, would select the elements from the cited prior art references for combination in the manner claimed." *Northern Telecom Inc. v.*

Datapoint Corp., 15 USPQ2d 1321, 1323 (Fed. Cir. 1990)

A *prima facie* case of obviousness requires the Examiner to cite to a combination of references which (a) suggests or motivates one of skill in the art to modify their teachings to yield the claimed invention, (b) discloses the elements of the claimed invention, **and** (c) provides a reasonable expectation of success should the claimed invention be carried out. Failure to establish **any** one of these requirements precludes a finding of a *prima facie* case of obviousness and, without more, entitles Applicants to withdrawal of the rejection of the claims in issue.[1] Applicants urge that the Examiner has failed to establish not one, but **all three** requirements as discussed below.

The Deweer/McPherson combination

Deweer *et al.* is silent on the modification of a *Bacillus* pullulanase generally and to the specific modifications currently claimed. McPherson *et al.* is similarly silent on the modification of a *Bacillus* pullulanase generally and to the specific modifications currently claimed.

The combination fails to suggest or motivate one of skill in the art to modify the teachings to yield the claimed invention

As noted above, McPherson *et al.* at best teaches that there are five discreet "amylase" regions conserved between amylases and a single, non-*Bacillus* pullulanase. The conserved "amylase" sequences are not provided by McPherson *et al.* so an alignment is not easily accomplished, if at all. There is no mention by McPherson *et al.* of either the Y or VWAP regions as required by the current claims. Therefore, a skilled artisan would find no reason to combine Deweer *et al.* with McPherson *et al.*, even if they were to combine the references the combination would not yield the currently claimed invention.

The combination fails to disclose the elements of the claimed invention

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[1] *See e.g., Northern Telecom Inc. v. Datapoint Corp.*, 15 USPQ2d 1321, 1323 (Fed. Cir. 1990); and *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988).

The combination fails to disclose the elements of the claimed invention

The currently presented claims are directed to: (a) a truncated *Bacillus* pullulanase comprising (b) a deletion of about 100 amino acids from the amino terminus of a pullulanase (c) obtainable from *Bacillus deramificans*, wherein said truncated pullulanase comprises (d) a conserved Y region, and (e) is capable of catalyzing the hydrolysis of an alpha-1, 6-glucosidic bond.

As noted above, McPherson *et al.* fails to mention either of the Y or VWAP regions. Furthermore, there is no mention of a deletion of about 100 amino acids from the amino terminus of a pullulanase obtainable from *Bacillus deramificans* in either Deweer *et al.* or McPherson *et al.* Although McPherson *et al.* describes a deletion of 170 amino acids that is in an unrelated gram-negative bacterial pullulanase, not in a Bacillus pullulanase. Therefore, the combination fails to disclose at least two of the elements of the presently claimed invention.

The combination fails provide a reasonable expectation of success

As previously stated, a skilled artisan would not have a reasonable expectation of success if they were to combine the references. First, there is nothing in Deweer *et al.* that indicates that a truncation of 98, 100, 102, 200 or 300 amino acids would result in an enzyme capable of catalyzing the hydrolysis of an alpha-1, 6-glucosidic bond.

Although McPherson *et al.* does teach a 170 amino acid deletion in an unrelated pullululanase there is no information provided that would allow the skilled artisan to perform a sequence alignment with a *Bacillus* pullulanase to know whether or not a similarly large deletion would work. Notably, the *Klebsiella* pullulanase of McPherson *et al.* is 120 kD whereas a *Bacillus* pullulanase is smaller. A similarly large deletion in the smaller enzyme may not work. Furthermore, there is no teaching that an even larger deletion would work. Thus, while there may be an invitation to try the deletion (that is not the standard; it is a standard that is frowned upon) there is no reasonable expectation of success.

For the foregoing reasons, the combination of Deweer *et al.* and McPherson *et al.* is inappropriate and fails to render the present invention obvious. Withdrawal of the rejection is respectfully requested.

The Deweer/Albertson combination

Deweer *et al.* is silent on the modification of a *Bacillus* pullulanase generally and to the specific modifications currently claimed. Albertson *et al.* is similarly silent on the modification of a *Bacillus* pullulanase generally and to the specific modifications currently claimed.

The combination fails to suggest or motivate one of skill in the art to modify the teachings to yield the claimed invention

Deweer *et al.* has been addressed *supra*. Albertson *et al.* is directed to a pullulanase from an extreme thermophile microorganism with approximately 35% identity with a different *Bacillus* pullulanase. In addition, Albertson *et al.* noted on page 38 (paragraph bridging columns) there was no three-dimensional structure for a pullulanase. As a point of reference, Applicants direct the Examiner's attention to Mosimann *et al.*, PROTEINS: Structure, Function and Genetics, vol. 23, pp. 301-317 (1995) (first page submitted herewith), wherein the author indicates that where sequence identity between the target and the template is greater than 70%, comparative molecular modeling is highly successful. Conversely, when "sequence identity is low (~30%)" modeling and energy minimization techniques fail to help. Mosimann *et al.* state "Based on these results it appears that ... deletions are still major problems." (See attached abstract). Given the low level of identity between what Albertson *et al.* reported as the most similar enzyme (~35%), the fact that *Bacilli* are not thermophilic organisms and the state of the art at the time (as evidenced by Mosimann *et al.*), one skilled in the art would not be motivated to apply the teaching of Albertson *et al.* to the *Bacillus* pullulanase of Deweer *et al.*

The combination fails to disclose the elements of the claimed invention

As noted above, the currently presented claims are directed to: (a) a truncated *Bacillus* pullulanase comprising (b) a deletion of about 100 amino acids from the amino terminus of a pullulanase (c) obtainable from *Bacillus deramificans*, wherein said truncated pullulanase comprises (d) a conserved Y region, and (e) is capable of catalyzing the hydrolysis of an alpha-1, 6-glucosidic bond.

Applicants contend the combination of references does not contain a teaching of a truncated pullulanase from a *Bacillus* species wherein the truncated enzyme retains the capacity to hydrolyze alpha-1,6-glucosidic bonds. Deweer *et al.* and Albertson *et al.* taken together do not disclose (b) a deletion of about 100 amino acids from the amino terminus of a pullulanase nor (d) a conserved Y region.

There is no teaching in either Deweer *et al.* or Albertson *et al.* that a *Bacillus* pullulanase could comprise a deletion of about 98, 100, 102, 200 or 300 amino acids from the amino terminus. If one were to use the alignment provided by Albertson *et al.* in Figure 2 (page 37 of Albertson *et al.*) for *Caldicellulosiruptor saccharolyticus* and *Bacillus acidopullulolyticus*, the 95 amino acid deletion in *C. saccharolyticus* would correspond to amino acid residue 137 in *B. acidopullulolyticus*. At best, this is an invitation to try, an inappropriate test for obviousness.

As taught by the Applicants at page 12 of the specification, Albertson *et al.* reveal the regions called DPY, A, B, C, D, E, and YNWGY as conserved regions among a group of gram-positive and gram-negative pullulanases. Two regions, DPY and YNWGY were identified as being characteristic of true pullulanases (although Applicants note that the *Thermus* sp AMD33 pullulanase shown in Figure 2 on page 37 lacks a DPY region). In addition to the conserved regions highlighted by Albertson *et al.*, Applicants significantly disclose two other conserved regions closer to the N-terminus of pullulanase. These regions are referred to as Y and VWAP and reference is made Figures 2A – 2D of the specification. Interestingly, the VWAP region, although clearly shown in Figure 2 on page 37 of Albertson *et al.* is not taught or suggested by Albertson

et al., as being a region of any importance. Applicants further disclose that the limits of amino acid truncations in the N-terminus of pullulanase would not go beyond the Y region, a small region (i.e., less than 6 amino acids in length) that was also not commented on by Albertson *et al.*

Therefore, the cited art combination fails to teach or disclose the claimed invention.

The combination fails provide a reasonable expectation of success

Reasonable expectation of success is assessed from the perspective of the person of ordinary skill in the art. See *Micro Chem.*, 103 F.3d at 1547, 41 U.S.P.Q.2D (BNA) at 1245.

A prior art reference must be considered in its entirety, *i.e.*, as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1550, 220 USPQ 303, 311 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). Prior art teaching away from a claimed invention cannot render the claimed invention obvious. *In re Haruna*, 249 F.3d 1327, 1335-36, 58 USPQ2d 1517, 1522 (Fed. Cir. 2001) ("'A reference may be said to teach away when a person of ordinary skill, upon reading the reference, . . . would be led in a direction divergent from the path that was taken by the applicant.' *Tec Air, Inc. v. Denso Mfg. Mich. Inc.*, 192 F.3d 1353, 1360, 52 USPQ2d 1294, 1298 (Fed. Cir. 1999).").

Applicants once again direct the Examiner's attention to Mosimann *et al.*, PROTEINS: Structure, Function and Genetics, vol. 23, pp. 301-317 (1995) (first page submitted herewith), and the discussion presented above regarding this piece of prior art. For the reasons provided above, one skilled in the art would not have a reasonable expectation of success in applying the teachings of Albertson *et al.* to the *Bacillus* pullulanase of Deweer *et al.* because of the low sequence identity and the lack of a tertiary structure as disclosed by Albertson *et al.*

Claim 12

The Examiner has rejected claim 12 as allegedly obvious over of Deweer, *et al.*

(US Pat. No. 6,074,854). The Examiner asserts that the cited reference teaches the claimed invention Applicants respectfully traverse.

Although Applicants must respectfully disagree with the Examiner's argument and rationale, in order to further the prosecution of the present application and Applicants' business interests, yet without acquiescing to the Examiner's arguments, Applicants have amended Claim 12 to more clearly recite that the pullulanase has one additional amino acid.

Withdrawal of the rejection is respectfully requested.

CONCLUSION

In light of the above amendments, as well as the remarks, the Applicants believe the pending claims are in condition for allowance and issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 846-7615.

Respectfully submitted,

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